THE CHROMOSOMES IN THE OÖGENESIS, FERTILIZATION AND CLEAVAGE OF COREID HEMIPTERA.

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I. Introduction.

The recent conclusions regarding sex-production based on the nuclear dimorphism of the spermatozoa in the tracheates have involved certain assumptions regarding oögenesis and cleavage which, though made in conformity with many well-determined facts, are still in need of more adequate support from direct observations. The principal of these assumptions is that in the formation of the polar bodies, the diploid chromosome-groups of the female are reduced to haploid groups that are alike in all the mature eggs. Further, since the spermatozoa are of two sorts, the embryos produced would be correspondingly different and this difference should be apparent from a study of the embryonic nuclei. At present, however, the complete chromosome-cycle has been worked out in only two groups of tracheates, the phylloxerans and aphids. In the phylloxerans, Morgan ('08, '09) has traced the full history of the chromosomes through several generations and the combined observations of Stevens ('05*a*, '06*a*, 09) von Baehr ('08, '09) and Morgan ('09) practically completes the cycle in the aphids. The very recent observations of Boveri and Gulick ('09), though as yet published only in brief outline, show that in *Heterakis*, a nematode, the chromosome-cycle is similar to that assumed for many insects and bears the same relation to sex-production, while Baltzer ('08) has found that in sea-urchins, the conditions are the reverse of those in insects, the eggs being dimorphic and the spermatozoa all of one kind.

The present work[1] was undertaken with the hope of demonstrat-

[1]This problem was begun in the zoölogical laboratory of Columbia University and completed in the anatomical laboratory of the College of Medicine, Syracuse University. A part of the material was collected while occupying a John D. Jones Scholarship room at the biological laboratory, Cold Spring Harbor, N. Y., during the summer of 1907, and a Wistar Institute room at the Marine Biological Labor

ing the chromosome groups in the oögenesis, fertilization and cleavage in certain coreid hemiptera and of determining in this way, if possible, whether the assumptions made in regard to the number and behavior of the chromosomes in these stages is in accordance with the facts.

Four species of the Coreidæ were examined: *Archimerus alternatus* Say, *Anasa tristis* De Geer, *Protenor belfragei* Hagl., *Chelinidea vittigera* Uhler.[2] In all of these forms the spermatogonia have been found to contain an odd number of chromosomes, one of which (the unpaired idiochromosome[3]) fails to divide in one of the maturation divisions. One half the spermatozoa thus contain this chromosome, the other half lack it, and a dimorphism of the spermatozoa arises. The oögonia have been shown to have an even number of chromosomes, the unpaired element of the spermatogonia being represented here by a pair of equal size. The maturation of the egg had not been fully worked out, but it was assumed that every chromosome divides equally in both polar divisions, giving to the mature egg a group of chromosomes similar to that borne by a spermatozoön having the idiochromosome. The eggs were accordingly assumed to be all of one kind with respect to their chromatin content, as direct observation has shown to be true in phylloxerans (Morgan), aphids (Stevens, von Baehr) and more recently in *Heterakis* (Boveri and Gulick.)

atory, Woods Hole, Mass., in 1909. To the directors of these laboratories I am indebted for the facilities placed at my disposal. I wish also to express my gratitude to Professors E. B. Wilson and T. H. Morgan for the many helpful suggestions they have made during the progress of this work.

[2]I am indebted to Mr. E. P. Van Duzee, of Buffalo, N. Y., for the identification of my material. The species *Archimerus alternatus* is almost, if not quite, identical with the *A. calcarator* of Professor Wilson's material (identified by Mr. P. R. Uhler) and was collected from the same locality in Van Cortlandt Park, New York. *Protenor* was found at Woods Hole, Mass., and *Anasa* at Woods Hole, and Cold Spring. Harbor, N. Y. *Chelinidea* was taken by Professor Wilson at Southern Pines, N. C. A part of the living specimens of *Anasa* were also furnished by Professor Wilson

[3]For the sake of simplicity, the term "idiochromosomes" will be used in this paper to designate those chromosomes which are associated with sex-production, irrespective of their detailed behavior in the growth periods or maturation divisions. It will thus include the "idiochromosomes" (in the more restricted sense), "accessory," "odd" or "heterotropic" chromosomes, "monosomes," "heterochromosomes," "X- and Y-elements," etc., of recent writers. Professor Wilson has used this term in a similar sense in the fourth of his "Studies" ('09*b*).

It was further supposed that if an egg is fertilized by a spermatozoön bearing the idiochromosome an embryo will result whose nuclei all have an even number of chromosomes, similar to the oögonial groups, but if fertilized by a spermatozoön lacking that chromosome, the embryonic nuclei will have an odd number of chromosomes similar to the spermatogonial groups. Accordingly the former will be females, the latter males. It should be possible, then, to distinguish the sex of an embryo by an inspection of the embryonic (somatic) chromosome groups. Again, if the number of chromosomes in the male and female pronuclei could be accurately determined just before the first cleavage spindle is formed, it would afford additional evidence of the dimorphism of the spermatozoa and the relation this condition bears to sex-production.

II. Material and Methods.

The insects were brought into the laboratory or greenhouse and placed in cages in which their food plants were growing. Here they paired readily and laid their eggs either on the plants or on the sides or bottom of the cages. The breeding periods of the four genera employed differ widely. *Anasa* may be found pairing on squash plants in the vicinity of New York or Woods Hole early in July, the eggs being laid in clusters on the under surfaces of the leaves, but specimens kept in a greenhouse over the winter laid early in May. *Chelinidea*, brought from the south and kept in a greenhouse during the winter, began to lay its clusters of eggs in the latter part of March. *Archimerus* begins laying on the goldenrod in the vicinity of New York in the latter part of May or first of June. The eggs are laid singly, and it was found impossible to collect sufficient numbers in the field so that all those used were taken from caged individuals. *Protenor* also lays its eggs one at a time and, in the laboratory, rarely makes any attempt at fastening them to any object, but drops them to the bottom of the cage where they were collected in small quantities. In addition to these, a number of eggs were taken from the oviducts of *Anasa* and *Protenor*.

The eggs of the four species differ markedly in size, *Archimerus* having the largest and *Protenor* the smallest, those of *Anasa* and

Chelinidea being intermediate; these size differences correspond roughly with the difference in size of the several species. All the eggs, whether in the oviduct or after laying, are enclosed in a tough brown chorion.

Several different fixing fluids were tried. Flemming's strong fluid, Gilson's mercuro-nitric and Bouin's picroformol were found very uncertain in result, as they seldom penetrate the thick chorion. All these can be used, however, if the eggs are pricked with fine needles before placing them in the fixing fluid, but their action is such as to render the yolk very brittle and difficult to cut. By far the best results were obtained by placing the eggs immediately in the Gilson-Carnoy acetic-alcohol-chloroform-sublimate mixture for fifteen to thirty minutes or in a mixture of glacial acetic, one part, absolute alcohol saturated with sublimate, two parts, for five to ten minutes. After either fluid, the eggs were transferred to iodized 95 per cent. alcohol for twelve hours and preserved in 80 per cent. alcohol. The acetic-alcohol-sublimate mixture was found invaluable for the earliest stages of maturation which occur while the eggs are still in the lower part of the oviduct and directly after laying. For later stages, the Gilson-Carnoy mixture gave excellent results. After immersion in alcohol, the egg shrinks away from the chorion which can then be removed with fine forceps and cutting needle. After removing the chorion, the eggs were dehydrated, cleared in cedar oil and immersed in melted paraffin for two hours. They were then oriented in a drop of paraffin and embedded. Serial sections were cut 6-8 μ thick on a sliding microtome. Very good series can be obtained in this way though the yolk sometimes becomes brittle and troublesome. The stain most frequently employed was iron-hæmatoxylin with or without a counter-stain. In addition to the eggs, ovaries and testes were fixed in Flemming's strong fluid and stained in iron-hæmatoxylin or safranin.

About twelve hundred eggs in all were sectioned, but owing to mechanical difficulties in technique, only about two hundred of these were of any value for study. In the maturation and fertilization stages particularly, one or two poor sections may render an entire series worthless, though in later embryonic stages this difficulty is not so serious. For this reason the results are

necessarily somewhat meagre, but they are perfectly clear as far as they go.

III. Descriptive.

A. *Oögenesis.*

The results on oögenesis are confined to the chromosomes of the oögonial and oöcyte divisions. No attempt has been made to trace the full history of the growth period, but an examination of a few eggs taken from the ovarian tubes seems to show that no definite chromatin-nucleolus or persistent oögonial chromatin element is present, as stated by Wilson ('06). The nucleus at this time contains many faintly staining chromatin threads and several small nucleoli whose nature was not determined. Foot and Strobell ('09) have found the same condition to be true in *Euschistus*, a pentatomid.[4]

1. *Archimerus alternatus.*

The spermatogonial groups[5] have been figured by Wilson in the second of his "Studies" ('05*b*), but for the sake of comparison two more are shown (Fig. 1, *c* and *d*). Each group has 15 chromosomes, two of which, the *m*-chromosomes (following Wilson's terminology), can always be identified by their very small size. Of the remaining thirteen no one can be positively identified, by its size or shape, as the idiochromosome. In the spermatocyte divisions this chromosome passes undivided to one pole of the spindle in the *first* mitosis and divides equally in the second (Wilson, '05*b*). This condition is peculiar to *Archimerus* alone of all the Coreidæ so far described,[6] but a rëexamination of the spermatocyte stages in new material shows beyond doubt that Wilson's account is correct. The idiochromosome can be easily identified in the first mitosis by its peripheral position on the spindle and by its unconstricted contour when the other chromosomes are in early, and even late, anaphase. It passes to one pole of the spindle a little behind the others. Further, in the

[4]Stevens ('06*b*) has described "heterochromosomes" in certain stages of the growth period of the oöcytes of *Aphrophora*, an homopteran.

[5]See footnote 2, at bottom of page 80.

[6]Professor Wilson has also found a similar condition in *Pachylis gigas* (unpublished).

cysts of second spermatocytes, one finds metaphase groups with eight and seven chromosomes side by side.

The oögonial groups[7] have not been figured previously, but Wilson gives the number of chromosomes as 16 in the fourth of his "Studies" (09*b*). In Fig. 1, *a* and *b*, two plates are shown

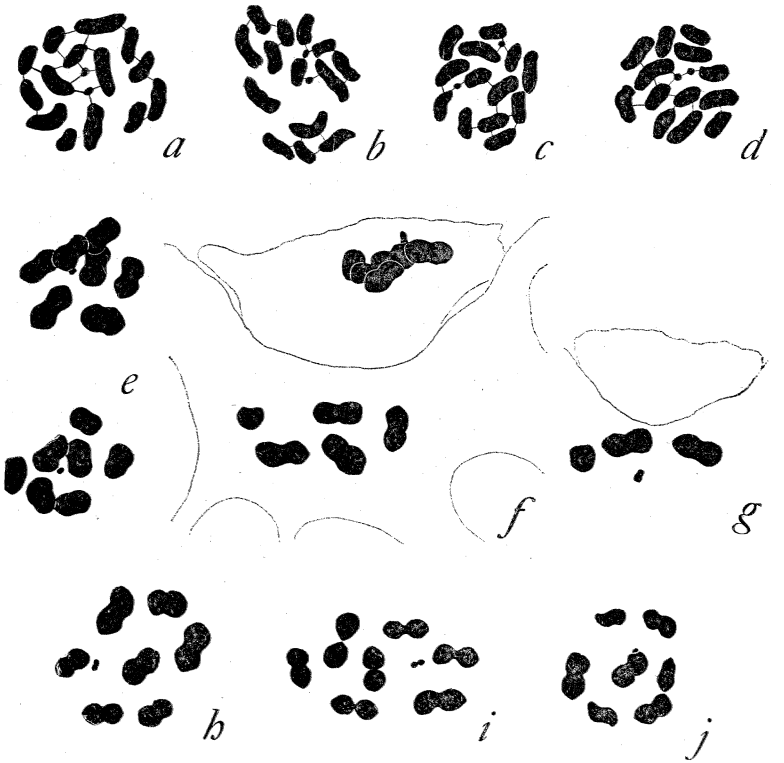

FIG. 1. *Archimerus alternatus*. *a* and *b*, oögonial, *c* and *d*, spermatogonial metaphase-groups. *e–j*, first oöcyte-(polar) division; *e*, daughter groups from the same anaphase-spindle, polar view; *f* and *g*, anaphase, side view, in two sections; *h* and *i*, inner daughter groups, polar view, of two anaphases; *j*, outer daughter group, polar view, of an anaphase.

each with 16 chromosomes. A pair of *m*-chromosomes again appears in each group. The fourteen larger elements present a somewhat graded series and cannot be paired off readily. Two

[7]The unreduced female groups in all the species, were taken from the region in the ovary lying just below the nutritive chamber, where oögonia, follicle-cells and young oöcytes are found together. For convenience they will be called "oögonia."

of the largest are probably the idiochromosomes, though they do not differ sufficiently from the others, in size or shape, to admit of exact identification.

The anaphase of the first mitosis was the earliest oöcyte stage obtained and was found in eggs just after laying. Fig. 1, *e* (from two sections), shows a polar view of this stage in which eight chromosomes can be accurately counted in both outer and inner groups. There are seven large dyads and one very small one in each, corresponding in relative size to the fourteen large and two small chromosomes of the oögonia. Fig. 1, *f* and *g*, show a side view of a late anaphase from two sections. The inner group contains eight dyads; one of them has divided prematurely, the two parts appearing in neighboring sections. The chromosomes in the outer group are too crowded to be counted. Pl. I., *a*, shows another anaphase in which the inner group is complete in one section and contains eight dyads. Fig. 1, *h* and *i*, show the inner groups of two more anaphases, polar view. The chromosomes in both are all dyads, eight in each, the component parts of which show all degrees of separation. This premature division of the dyads is very common in the final anaphases of the first division and might lead to mistakes in counting if it were not that one finds all stages of division up to the complete separation shown in Fig. 1, *f* and *g*. Henking found this condition in the egg of *Pyrrhocoris* ('92, Pl. III.) and it has also been found in the first spermatocytes of *Aphrophora* an homopteran (Stevens, '06*b*) and *Anax* a dragon-fly (Lefevre and McGill, '08). It is probable that even in cases of extreme separation, the halves of the dyads remained connected by fine strands of chromatin or linin which become invisible after long extraction of the stain. The second division is thus foreshadowed, in the anaphase of the first, and even before as will be shown in *Anasa*. Since there is no period between the first and second divisions when the chromosomes lose their individual contour, in fact no telophase in the strict sense, the dyads pass practically unchanged into the second maturation spindle.

The chromosomes which enter the first polar body retain their contour and grouping for some time, forming a flat plate when seen in surface view. Fig. 1, *j*, shows such a plate with seven

large chromosomes all more or less constricted and a small nodule close to the central one, which probably is the *m*-chromosome. Fig. 2, *b*, shows another in somewhat oblique view with seven large dyads, and the *m*-chromosome dyad, the latter faintly stained. Fig. 1, *f*, and Pl. I., *a*, show side views of two more polar body groups; in Fig. 1, *f*, the *m*-chromosome dyad is dis-

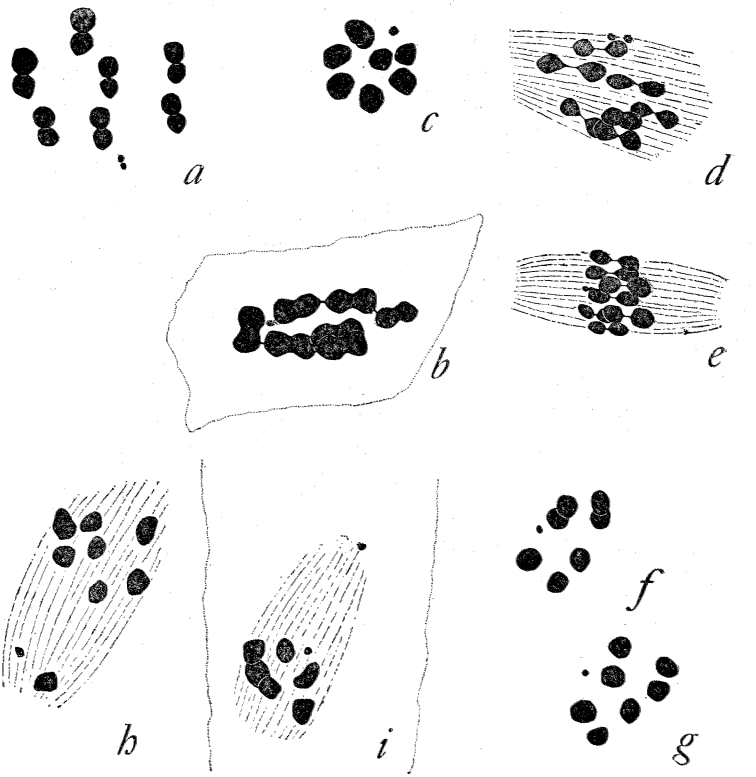

FIG. 2. *Archimerus alternatus*. Second oöcyte-(polar) division. *a*, metaphase-group, oblique view. *b*, chromosome-group of the first polar body from the same egg as the last. *c*, metaphase-group, polar view. *d* and *e*, metaphase groups, side view. *f* and *g*, daughter groups from the same anaphase-spindle, oblique view. *h* and *i*, anaphase, oblique view, in two sections.

tinctly seen. All the chromosomes show the typical constriction as though ready for a second division. However no spindle is formed and no division takes place except that in individual chromosomes the halves of a dyad may separate of their own accord,

just as in those of the inner groups. At the close of the second division the chromosomes of the first polar body are finally merged together in a deeply-staining mass.

The second division follows closely upon the first as stated above. The chromosomes do not crowd together in the final anaphase of the first division as in the spermatocytes, but merely rotate about forty-five degrees and become disposed upon a new spindle which forms out of the enveloping cytoplasm. Fig. 2, *a*, shows a metaphase of the second division in slightly oblique polar view. The seven large dyads and *m*-chromosome dyad are sharply constricted and ready for division. The first polar body with its eight dyads taken from the same section is shown in Fig. 2, *b*. Fig. 2, *c*, shows another polar view of a second division metaphase, and Fig. 2, *d* and *e*, are side views of the same, each showing eight chromosomes. All the dyads are more or less drawn out in preparation for division, and in anaphase, separate into two groups of monads. Fig. 2, *f* and *g*, show outer and inner groups respectively taken from the same anaphase; each has eight monads. Fig. 2, *h* and *i*, show an oblique view of an anaphase in two sections (the surface of the egg is indicated by a dotted line at the right of each section). The outer group which passes into the second polar body is shown above, the seven large monads in *h*, the *m*-chromosome monad in *i*. The inner group which remains in the egg, is shown below; six of the large monads and the *m*-chromosome monad in *i*, the remaining large monad in *h* (The stippled object in *h* is part of one of the chromosomes in *i*). Thus the end result is, that the female pronucleus is formed from eight single elements or monads, comparable to those borne by a spermatozoön containing the idiochromosome (vid. Wilson, '06).

There are no peculiar or "lagging" chromosomes in either division. Which of the polar divisions is reducing, *i. e.*, separates whole chromosomes which have paired in synapsis, could not be determined, since the first oöcyte prophases were not obtained.

While it is not possible to identify the idiochromosomes in the two divisions just described, the *m*-chromosome appears as a constant element dividing in both. In the spermatocytes Wil-

son's ('05*b*) figures show it occupying the centre of spindle in both divisions. However, in the oöcytes its position is variable in the first division (Fig. 1, *e*, *h*, and *i*; Pl. I., *a*) but always peripheral in the second (Fig. 2, *a*, *c*, *d*, *f* and *g*, *h* and *i*).

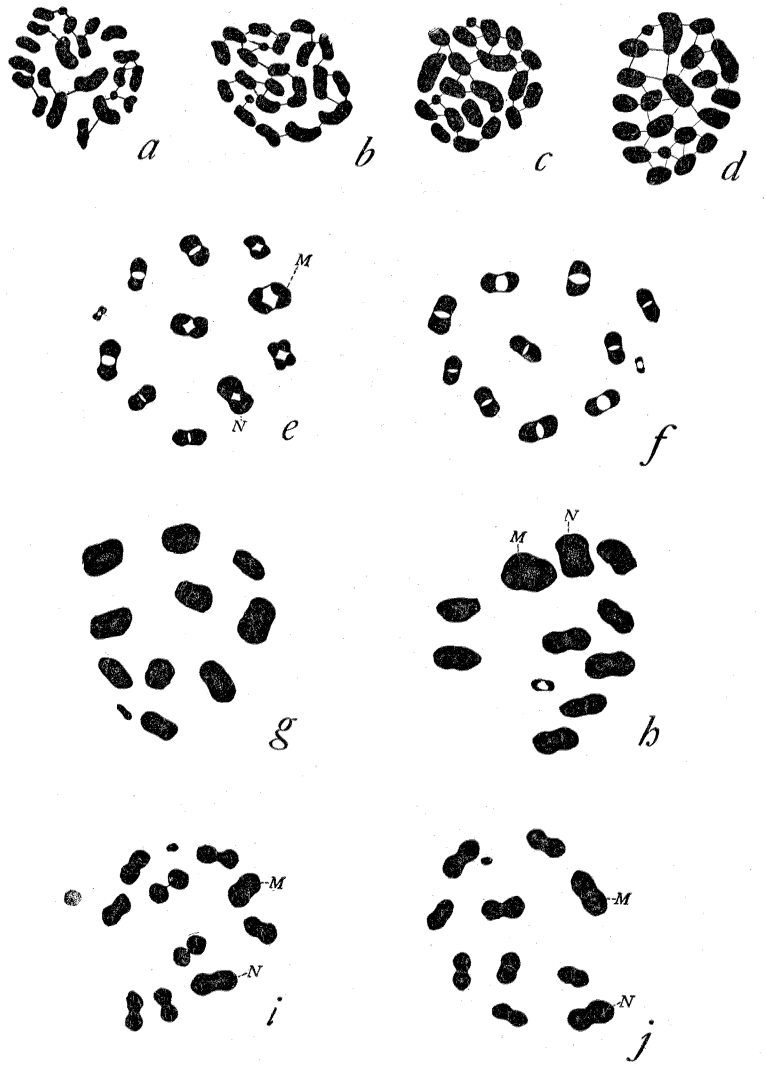

FIG. 3. *Anasa tristis*. *a* and *b*, oögonial, *c* and *d*, spermatogonial metaphase-groups. *e–j*, first oöcyte-(polar) division: *e*, *f*, *g* and *h*, metaphase-groups, polar view. *i* and *j*, daughter groups from the same anaphase-spindle, polar view.

2. *Anasa tristis.*

The spermatogonial groups have been figured by Wilson ('05*b* and '06), and the number of chromosomes stated as 21. Wilson's count has been corroborated by Montgomery ('06) and by Lefevre and McGill ('08) though Paulmier ('99) and Montgomery in an earlier paper ('01) had given the number as 22. Foot and Strobell ('07*a* and 07*b*) have disputed the twenty-one count and, on the basis of photographs of smear preparations, have concluded that the number is 22, confirming Paulmier's original account. A reëxamination of fresh material from two different localities gave 21 as the correct number of spermatogonial chromosomes in agreement with accounts of Wilson, Montgomery, and of Lefevre and McGill. Imperfect plates it is true may show less than this number, but none were found with more. In *Chelinidea vittigera*, where the spermatogonial groups are almost identical with those of *Anasa*, a single exception may be cited, in which 22 chromosomes were found. Apart from this, all clear plates gave 21.

As pointed out by Wilson and by Lefevre and McGill, the spermatogonial groups of *Anasa* show two *m*-chromosomes and three largest chromosomes one of which must be the unpaired chromosome ("heterotropic" or "accessory" chromosome) though it cannot be precisely identified by its size or shape. Fig. 3, *c* and *d*, shows two spermatogonial groups illustrating these points.

The oögonial groups (Fig. 3, *a* and *b*) contain 22 chromosomes, including two *m*-chromosomes and four largest chromosomes. Of the last named, two must be the idiochromosome pair, corresponding to the unpaired idiochromosome of the spermatogonia, as pointed out by Wilson in the third of his "Studies" ('06).

The metaphase of the first oöcyte division was found in eggs taken from the lower part of the oviduct. Fig. 3, *e*, *f*, *g* and *h*, shows four such phases, *h* being a composite figure from two sections.[8] There are 11 chromosomes in each, all undoubtedly

[8]The difference in size between the chromosomes as a whole of Fig. 3, *e* and *f*, and those of Fig. 3, *g* and *h*, is probably due to a difference in the length of fixation. In the former case the eggs were left for ten minutes in the fixative (alcohol-acetic-sublimate), in the latter, for five minutes. The chromosomes of Fig. 3, *g* and *h*, seem to show the swelling action of the acetic unchecked by the sublimate which penetrates more slowly.

FIG. 4. *Anasa tristis*. *a–f*, first oöcyte-(polar) division; *a*, four chromosomes in initial anaphase, side view, showing tetrad-character; *b*, final anaphase, side view; *c* and *d*, daughter groups from the same anaphase-spindle, slightly oblique polar view; *e* and *f*, the same from another egg; *g*, metaphase-group, side view, second oöcyte-(polar) division. *h*, the chromosomes of the last drawn at three different focal levels. *i*, the chromosome-group of the first polar body from the same eggs

double, arranged in an irregular ring with one in the centre and one or two outside. Unlike the first spermatocytes, the central chromosome is not the *m*-chromosome bivalent but one of the larger ones, while the *m*-chromosome itself lies just within the ring (Fig. 3, *e*, *f* and *h*) or somewhat outside (Fig. 3, *g*). In these four metaphases, the axis of the spindle is somewhat oblique to the plane of section and the structure of the chromosomes can thus be readily observed. As might be expected many have the shape of typical tetrads foreshadowing the two oöcyte divisions. This is especially well seen in Fig. 3, *e*, where even the *m*-chromosome has the quadripartite form. Whether distinctly quadripartite, or simply dumb-bell shaped, the plane of the first division is clearly indicated in every chromosome. In Fig. 3, *e* and *h*, there are two bivalents (*M* and *N*) perceptibly larger than any of the others. In all probability these arise from a pairing of the four largest chromosomes of the oögonia. Accordingly one of them may be considered to be the idiochromosome bivalent.

The anaphase of the first polar spindle was found in eggs directly after laying.[9] The chromosomes divide in the plane already indicated at metaphase. Fig. 4, *a*, shows a side view of four chromosomes in initial anaphase; the tetrad character of each is clearly indicated. Fig. 3, *i* and *j*, shows polar views of the outer and inner groups respectively from the same spindle; each has 11 chromosomes. All, with the exception of the *m*-chromosome in *j*, are obviously dyads showing all degrees of constriction, as in the first polar anaphase of *Archimerus*. The outer group (*i*) which passes into the first polar body shows two dyads completely divided though the polar body itself neither divides nor forms a spindle. In both groups two largest dyads (*M* and *N*) can be distinguished. Fig. 4, *c* and *d*, are outer and inner groups respectively from another spindle, in slightly oblique polar view (in *c* the large chromosome, *N*, was found in the same section as the inner group, *d*). Both groups contain 11 dyads of which two (*M* and *N*) are larger than any of the others. Fig. 4, *e* and *f*, are outer and inner groups respectively of a third anaphase in

[9] A single exception to this was found. One egg taken from the lowest part of the oviduct showed the first polar spindle in initial anaphase. It is possible that rough handling started the maturation process, as has been found in other groups of animals.

somewhat oblique polar view (the entire outer group, *e*, and six chromosomes of the inner group, *f*, appeared in one section, the remaining five of the latter group, in the next section). Both groups contain 11 dyads of which several in *f* show premature separation of their parts.

The peripheral position assumed by the *m*-chromosomes in metaphase (Fig. 3, *e*, *f* and *g*) is again seen in all the anaphase groups (Fig. 3, *i* and *j*; Fig. 4, *c*, *d*, *e* and *f*). This is undoubtedly their normal position in the first polar division. A side view of the final anaphase (Fig. 4, *b*) shows no peculiar or "lagging" chromosome on the spindle. All the chromosomes divide equally, as the polar views of three final anaphases show, where every chromosome is distinctly visible.

A single example of the second polar division was found (Fig. 4 *g*). It is a side view of a metaphase with 11 dyads. In *h*, the dyads of this group are drawn at three different focal levels. The first polar body (*i*) of the same egg also contains eleven dyads. In both the second polar spindle and the first polar body, there are two dyads (*M* and *N*) somewhat larger than any of the others. These are undoubtedly the products of division of the two largest tetrads of the first polar metaphase (Fig. 3, *e* and *h*). The *m*-chromosome in the second division (Fig. 4, *g* and *h*) takes a peripheral position as it did in the first. It appears also in the first polar body (*i*). In short there are three chromosomes distinguishable by their size, which can be identified in both polar divisions. These in all probability arise from a pairing of the four largest and two smallest oögonial chromosomes.

Though no anaphases of the second division were found, it is almost certain from the results in *Archimerus* that all the dyads divide equally, in a plane corresponding to the constriction shown at metaphase. Accordingly the female pronucleus, as well as the second polar body, will contain 11 monads and correspond to a spermatozoön bearing the idiochromosome as assumed by Wilson ('06).

3. *Protenor belfragei.*

The spermatogonial groups have been described and figured by Montgomery ('01 and '06) and by Wilson ('06). These observers agree that there are 13 chromosomes, one of which is

more than twice as large as the next in size. Of the remaining twelve, two are much larger than the others, and the rest form a graded series of pairs. The smallest pair, the *m*-chromosome, is relatively larger in this species than in *Archimerus* and *Anasa*. In Fig. 5, *c* and *d*, are shown two spermatogonial groups illus-

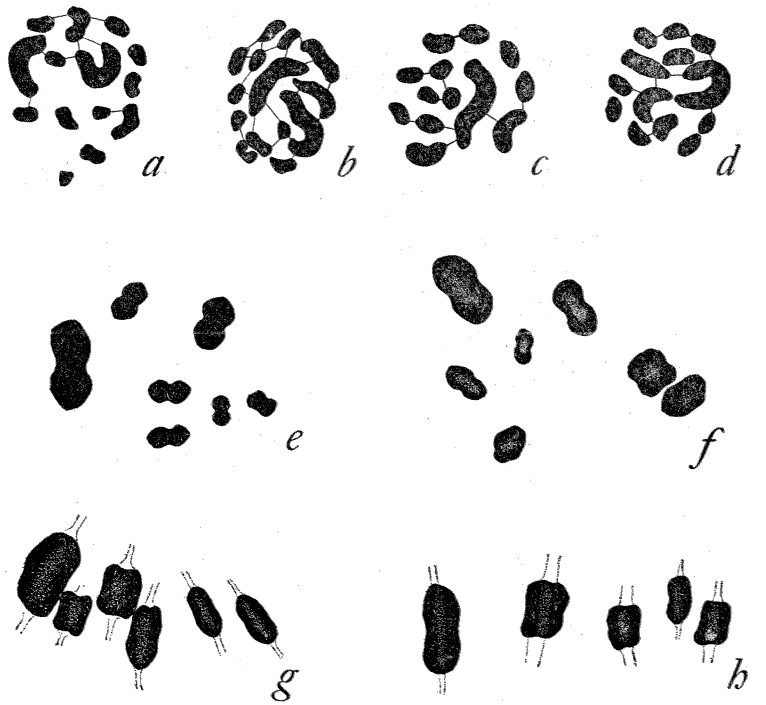

FIG. 5. *Protenor belfragei*. *a* and *b*, oögonial, *c* and *d*, spermatogonial metaphase-groups. *e–h*, first oöcyte-(polar) division; *e*, metaphase group, polar view; *f*, metaphase group, oblique polar view; *g*, six chromosomes in metaphase, side view; *h*, five chromosomes in metaphase, side view.

trating the above mentioned points. The idiochromosome does not appear constricted as figured by Montgomery ('01), Fig. 134.

The oögonial groups were figured by Wilson in the third of his "Studies" ('06). In them "there are two very large chromosomes, equal in size, in place of the single one that appears in the male, while the remaining chromosomes show the same relations as in the male." In Fig. 5, *a* and *b*, two of these groups are shown.

Of the maturation stages, only four preparations were obtained,

all of the first polar metaphase (Fig. 5, *e*, *f*, *g* and *h*).[10] Two of these (*e* and *f*) are complete, showing seven bivalents. The idiochromosome bivalent can be positively identified by its relatively enormous size, having been formed, no doubt, by the synapsis of the two largest chromosomes (idiochromosomes) of the oögonia. It does not in any way resemble a nucleolus. The bivalent next in size, corresponding to the two next largest chromosomes of the oögonia, can also be identified. Indeed the bivalents as a whole show the same relative size differences as the chromosome pairs in the oögonia. The *m*-chromosome bivalent is the smallest but only slightly smaller than the next in size. In Fig. 5, *f*, three chromosomes of intermediate size are seen in face view; two of these exhibit a quadripartite form, clearly indicating their bivalent nature. Fig. 5, *g*, is a side view of an incomplete metaphase, drawn from two sections, showing six of the seven chromosomes, and Fig. 5, *h*, shows a metaphase, side view with only five chromosomes, again taken from two sections. In both of these figures the idiochromosome bivalent can be readily identified by its size and the plane of the second division is clearly indicated in all the bivalents. While the further processes of maturation were not followed, it may be inferred, on the analogy of *Archimerus*, that in the anaphase of the first division the seven tetrads divide into two groups of dyads and the inner group of these separate in the second division into two groups of monads; the inner group of these last named, seven in number, enter into the formation of the female pronucleus, which is thus similar in chromatin-content to a spermatozoön bearing the idiochromosome.

4. *Conclusions Regarding Oögenesis.*

The results on maturation are somewhat meagre it is true but perfectly clear as far as they go, and point to the conclusion that, unlike the spermatozoa, all the mature eggs are of one kind with respect to their chromatin-content, as has been assumed. The female pronucleus contains a reduced group of chromosomes similar in size and number to that carried by a "female-pro-

[10]The excessive size of the chromosomes in these figures, especially those of *g* and *h*, is probably due to the peculiar action of the fixative (see footnote 8, on page 89).

ducing" spermatozoön, *i. e.*, one bearing the idiochromosome (directly proved in *Archimerus*, but only in part in *Anasa* and *Protenor*). The idiochromosome bivalent is not distinguishable by its behavior from other chromosomes, and divides in both mitoses, giving an equal portion to each oötid. It never assumes a nucleolus-like form either in the oögonial or oöcyte divisions.

B. *Some Details of Polar Body Formation.*

The place of polar body formation has been found to vary in different groups of insects. It may be on the dorsal surface, either approximately midway between the poles as in *Blatta* (Blochmann, Wheeler) and *Pyrrhocoris* (Henking), or a short distance behind the anterior end as in *Musca* (Blochmann, Henking). In Chrysomelidæ (Hegner) it is ventral, while in *Pieris* (Henking) it is close to the anterior (micropylar) end. In *Hydrophilus* (Heider) and *Aphis* (Stevens) it is lateral. In *Archimerus* the polar bodies are given off on one of the flat surfaces of the egg, approximately midway between the poles. In this species the two surfaces cannot be distinguished after the chorion has been removed, but in *Protenor* the dorsal surface is markedly convex while the ventral is flat or slightly concave, and it was here determined by proper orientation that the place of polar body formation is on the dorsal surface. The first polar spindle lies in a small thickening of cytoplasm with its axis at right angles to the egg surface (Fig. 1, *f* and *g*; Fig. 4, *a* and *b*; Pl. I., *a*). The anaphase of the first polar division occurs just after laying (in *Archimerus* a single exception was found; see footnote 9, page 91). No centrosomes or asters could be demonstrated by the method of fixation used just as Henking ('92) found in *Pyrrhocoris* though he used a different method. In late anaphase a cell-plate is formed by swelling of the spindle fibers and the surface of the egg dips down and around the outer group of chromosomes, until finally a little mass of cytoplasm containing the latter comes to lie free in a depression of the egg surface (Fig. 1, *f* and *g*; Fig. 4, *b*; Pl. I., *a*). In both the first and the second polar divisions (Fig. 4, *b*, and Pl. I., *b*) the constriction probably does not involve the cell-plate but passes between it and the outer group of chromosomes as Henking observed in *Pyrrhocoris*.

In *Anasa*, a rounded cytoplasmic body was found, in two cases, in or near the first polar spindle (Fig. 3, *i*; Fig. 4, *c-d*). This is perhaps comparable to the "eigenthümliches Körperchen" which Henking described in *Pyrrhocoris*. It may be a plasmosome, but it is difficult to decide, since one frequently finds a number of cytoplasmic bodies in the neighborhood of the first polar spindle (Pl. I., *a*) which cannot be distinguished from yolk granules and which are inconstant in appearance and number.

At the conclusion of the first polar division the spindle gradually fades away; there is no persistent cylindrical mass of spindle fibers or "thelyid" as Henking ('90 and '92) found in *Pieris*, a lepidopteran, and *Agelastica*, a coleopteran. The chromosomes left in the egg, as stated before, remain separate and there is no telophase in the strict sense. After a short resting period, they rotate about 45° and become disposed on a new spindle which has formed out of the cytoplasm surrounding them. The axis of the second polar spindle lies very obliquely to the surface of the egg (Fig. 2, *h* and *i*; Pl. I., *b*). As in the first division there are no centrosomes or asters. In late anaphase a cell-plate is formed by swellings of the spindle fibers. The second polar body is constricted off in the same manner as the first and lies alongside of it in the same depression. The first does not divide. The two bodies finally become embedded in the surface cytoplasm and can be distinguished as late as the third or fourth cleavage.

At the close of the second polar division, the chromosomes left in the egg become massed together and are converted into the female pronucleus (Pl. I., *b*). Those which have entered the polar bodies may remain separate for some time but eventually fuse into one or two deeply staining masses.

C. *Fertilization.*

The spermatozoa enter the egg through the micropyles which form a conspicuous ring at the anterior end. Polyspermy is undoubtedly normal, for accessory sperm nuclei were found in the egg as late as the copulation stage shown in Pl. II., *b*. As many as three of these nuclei appeared in some cases. At the time when the first polar spindle is in late anaphase, the sperm head enveloped in a mass of cytoplasm has moved some distance

into the egg among the yolk spheres leaving a train of cytoplasm behind it. It appears as a compact deeply staining rod surrounded by a clear area and preceded by an aster (Pl. I., *d*). The clear area is probably the "arrhenoid" mentioned by Henking ('92) in his account of *Pyrrhocoris*. The sperm head often appears coiled at the end which points away from the direction of its movement. Later it loses its staining power and opens out into an oval vesicle (Pl. II., *a*). The clear area and aster are here well marked though no centrosome is visible in the preparation. Still later, the vesicle becomes considerably larger and small irregular masses of chromatin can be seen in its interior. It is then ready for copulation.

In the meantime, the egg nucleus, formed from the inner group of chromosomes of the second polar spindle, has begun to move into the egg surrounded by a small mass of cytoplasm (Pl. I., *c*). The cytoplasm frequently contains one or more yolk spheres. The nucleus is at first round in outline and the chromatin is distributed in small nodules lying chiefly against the nuclear membrane. Subsequently it loses its capacity for staining, and appears somewhat like the sperm head, but more rounded. It then begins to increase in size, becoming at the same time irregular in shape and the chromatin once more appears in irregular masses.

As the two pronuclei approach each other their cytoplasmic areas fuse and they come to lie side by side with an amphiaster between (Pl. II., *b*; the aster on the upper side of the nuclei was drawn from the next section). In contact with each pronucleus, may often be seen a large clear vesicle. These probably represent the structures mentioned by Henking as the "descendants of the arrhenoids," *i. e.*, derived from the clear area surrounding the male pronucleus. In Pl. II., *b*, one of the asters contains a minute centrosome. The entire amphiaster is probably formed under the influence of the male pronucleus, for, in the same egg from which Pl. II., *b*, was taken, an accessory sperm nucleus was found with a very small amphiaster lying in contact with it. The further history of the clear vesicles could not be followed as very few first cleavage figures were found; at a later stage of copulation (Pl. II., *c*) they did not appear. Henking

described them in *Pyrrhocoris* as forming the poles of the first cleavage spindle ("Polkörperchen") and apparently considered them to be archoplasmic masses. That he did not see an aster in front of the male pronucleus nor an amphiaster at copulation, in addition to these structures, is perhaps due to his methods of technique.

During the approach of the pronuclei the chromatin in each becomes more and more condensed until the compact somewhat elongated chromosomes appear. Pl. II., *c*, the single example of this stage found, shows the pronuclei of *Archimerus*, still slightly separated. An indistinct aster appears at the right. In the lower nucleus seven chromosomes of different sizes can be distinctly seen. The *m*-chromosome is missing and because of its small size could not be identified in the next section. There are no nucleoli in either pronucleus.[11] The chromosomes in the upper nucleus are not yet fully condensed. The two pronuclei are so nearly equal in volume that one cannot distinguish which is male and which female even before copulation (Pl. II., *c*). It is apparent from a comparison of Pl. II., *b* and *c*, that both undergo a marked decrease in volume just before their nuclear membranes fade out. Pl. II., *d*, shows a late copulation stage or prophase of the first cleavage spindle of *Protenor* in polar view. The nuclear membranes have faded out but the chromosome groups derived from each pronucleus are still separate. This figure is obviously incomplete but it shows distinctly one reduced group (at the right of the figure) in which all the chromosomes appear, seven in number. Just as in the first oöcyte division, the idiochromosome is here recognizable by its relatively large size, and does not in any way resemble a nucleolus. A next largest chromosome and an *m*-chromosome also can be identified, the remaining four being intermediate in size. The group at the left of the figure shows the idiochromosome and three others, the remaining chromosomes being too crowded in the next section to identify. Since each group contains an idiochromosome it is not possible to say which was derived from the egg nucleus and which from a sperm of the class which bear this chromosome.

[11]Stevens ('06*a*, Pl. IV., Fig. 119) has figured this stage in the "Goumi aphid" where there are five chromosomes in each pronucleus, and, in the female, two plasmosomes in addition.

However, the embryo arising from this union would have been a female, for all the products of the first cleavage nucleus would contain two idiochromosomes as in the oögonia. A chromosome group taken from a female embryo is shown in Fig. 12, *c*. Even these meagre results make it probable that the chromosomes coming out of the male and female pronuclei at copulation are of the same number and show the same relative size differences as those which previously entered into the formation of the gametic nuclei.

D. *The Cleavage and Blastoderm Nuclei.*

The cleavage nuclei are formed by successive division of the fertilization nucleus. After each division the daughter nuclei move apart, each surrounded by a star-shaped cytoplasmic island. They wander toward the periphery, continually dividing by mitosis and there form the blastoderm. No instances of amitosis were observed in these stages such as Wheeler ('89) described in *Blatta*. Although but few first cleavage divisions were found, the chromosomes in them do not differ from those of somewhat later stages described beyond. The cleavage mitoses all show spindles, centrosomes and asters with diagrammatic clearness and the chromosomes, though somewhat elongated, can be counted as readily as in the oögonial or spermatogonial divisions. In metaphase each chromosome appears on the spindle split lengthwise and in anaphase the halves separate as in ordinary homotypic division. In telophase the chromosomes at either pole become vesicular, fuse together and form a daughter nucleus. At first the contents of the resting nucleus entirely lacks staining power, no nucleoli of any kind appearing. As the time for the subsequent division approaches, small flakes of chromatin appear which increase in number and gradually unite to form the chromosomes. In the cleavage stages, no definite chromatin nucleoli or plasmosomes could be seen with the methods of fixation employed, nor was there any elimination of chromatin during the earlier mitoses as described by Boveri in *Ascaris*.

1. *Archimems alternatus.*

A careful study of the eggs after fertilization revealed the fact that there are two sorts of embryos, one having 15 chromosomes

in all its cleavage—and blastoderm—nuclei and the other 16. These chromosome numbers are the same as those found in the spermatogonia and oögonia respectively. The size-relations also

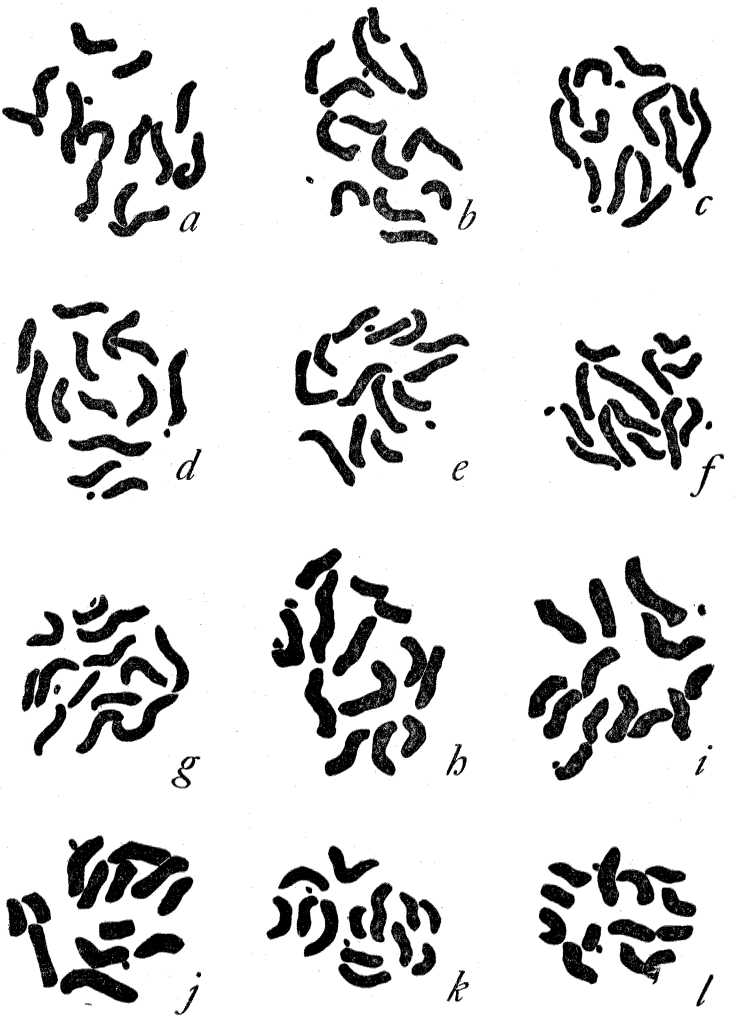

FIG. 6. *Archimerus alternatus.* Chromosome-groups of embryonic cells, 15-chromosome type. *a–e*, from the same embryo; *f–l*, from other embryos.

are in general the same as in the gonads. It seems fair then to conclude that the 15-chromosome embryos are males, the 16-chromosome, females. In Fig. 6 are shown twelve 15-chromo-

some groups taken from embryos at different stages. Fig. 6, *a*, *b*, *c*, *d* and *e*, are from the same embryo early in the formation of the blastoderm. Fig. 6, *f* and *g*, are from another embryo in the same stage. Fig. 6, *h* and *i*, are from an embryo in a slightly later stage of the blastoderm. Fig. 6, *j*, is from still another at the same stage as the last, and *k* from a late blastoderm. Fig. 6,

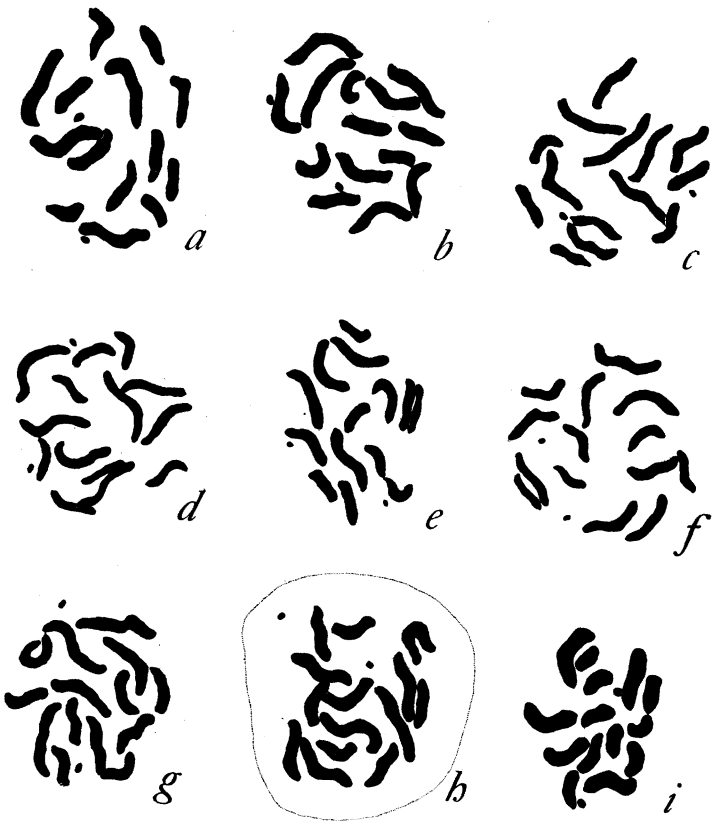

FIG. 7. *Archimerus alternatus*. Chromosome-groups of embryonic cells, 16-chromosome type. *d*, *e*, and *f*, from the same embryo; others from different embryos.

l is taken from a stage in which the blastoderm is invaginating to form the embryonic fundament and membranes. The 16-chromosome groups are shown in Fig. 7. Fig. 7, *a*, shows a group taken from the interior of an egg in the cleavage stage. Fig. 7, *b* and *c*, are from an embryo in the early blastoderm stage.

Fig. 7, *d*, *e* and *f*, are from another embryo in the same stage as the last. Fig. 7, *g* and *h*, are from a slightly later blastoderm and *i* is from a completed blastoderm.

An inspection of Figs. 6 and 7 as a whole, shows that in the earlier stages, the chromosomes are somewhat elongate (Fig. 6, *a–g*; Fig. 7, *a–f*) and that as development proceeds, they become shorter and thicker until at the time of invagination or just before (Fig. 6, *k–l*; Fig. 7, *i*) they have about the same contour as those of the spermatogonia and oögonia (compare with Fig. 1, *a–d*). In every stage the *m*-chromosomes, though very minute, are constant elements. The unpaired idiochrosome in the male groups and the paired idiochromosomes in the female cannot be distinguished by their size or contour but are probably represented among the larger chromosomes. The remaining chromosomes cannot be readily paired off.

2. *Anasa tristis.*

The embryonic mitoses of *Anasa*, though having a larger number of chromosomes that those of *Archimerus*, are much more favorable for making chromosome counts, especially in the early (incomplete) blastodern stage, *i. e.*, at a time when many of the cleavage nuclei have reached the surface and are still rapidly dividing. The embryos are of two classes: one having 21 and the other 22 chromosomes. Since these numbers correspond to those in the spermatogonia and oögonia respectively, it may be concluded that the 21-chromosome class are males, the 22-chromosome class, females. Fig. 8, *a–h*, show eight metaphase groups from the 21-chromosome class. Fig. 8, *a*, *b*, *c*, *d*, *e* and *f*, are taken from an embryo in the early blastoderm stage. In this embryo ten more perfectly clear groups were found each with 21 chromosomes, making sixteen in all from the same embryo. Fig. 8, *g* and *h*, are from another embryo in the same stage. In Fig. 9 six groups of the 22-chromosome class are shown, all from the same embryo in the early blastoderm stage.

One exceptional group was found in an embryo of the 22-chromosome class (Fig. 8, *i*). This group contains 23 chromosomes, of which three are larger than the rest. It is difficult to suggest an explanation for this condition. It may be due to an

accident of technique, the microtome knife tearing a chromosome in half as it passed through the block, or it may be the result of an abnormality in a previous division. There were no other

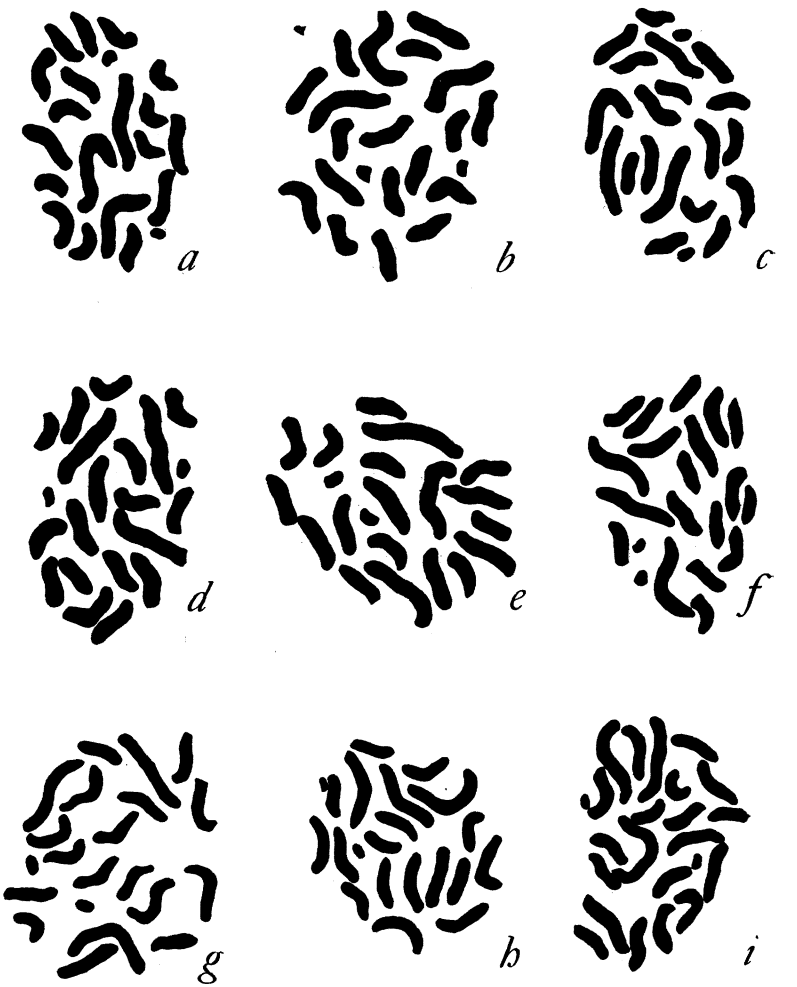

FIG. 8. *Anasa tristis*. Chromosome-groups of embryonic cells. *a–h*, 21-chromosome type: *a*, *b*, *c*, *d*, *e* and *f*, from the same embryo; *g* and *h*, from another embryo. *i*, exceptional group with twenty-three chromosomes.

mitoses in the immediate vicinity from which an extra chromosome could have been derived. The chromosome groups represented in Figs. 8 and 9 were selected from a large number of

very clear preparations. Many more could have been shown, but it seemed needless to multiply the number of figures.[12]

A comparison of the male groups (Fig. 8, *a–h*) with the female groups (Fig. 9) shows clearly that in the former there are three chromosomes larger than the rest, while in the latter there are four such elements. These size relations are the same as those in the spermatogonia and oögonia respectively (vid. page 89).

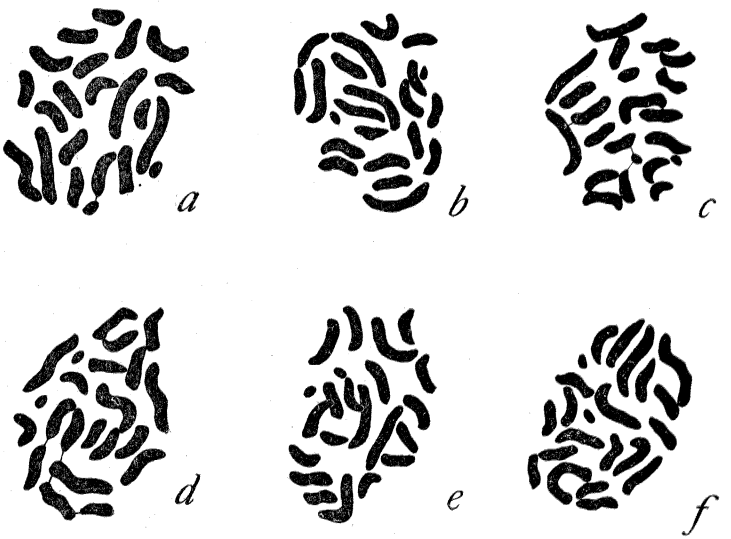

FIG. 9. *Anasa tristis*. Chromosome-groups of embryonic cells, 22-chromosome type, all from the same embryo.

Accordingly, one of the large chromosomes of the male groups must be the unpaired idiochromosome and two of the large chromosomes of the female groups, the paired idiochromosomes. The *m*-chromosomes appear as constant elements in both groups and are usually more elongated than in the germ-cells, a feature which is common to all the chromosomes. Apart from the largest and smallest elements, this elongated condition makes it impossible to pair off the remaining chromosomes with any degree of certainty.

[12]All the figures were drawn with camera lucida, Zeiss apochromat. 2 mm., compens. oc. 12. With the exception of Plates I. and II., they were again enlarged with the camera and subsequently reduced in reproduction one-half, giving a final magnification of 2,650 diameters. The magnification of Plates I. and II., is 1,375 diameters. Achromatic structures, except those of Plates I. and II., have been represented semi-schematically.

3. *Chelinidea vittigera.*

The embryonic groups of this species are very similar to those of *Anasa* and equally favorable for making chromosome counts. The embryos are again of two sorts, one having 21 chromosomes, the other 22. In fact the number and size-relations are so much like those in *Anasa* that the two forms cannot be distinguished by their chromosome complexes alone.

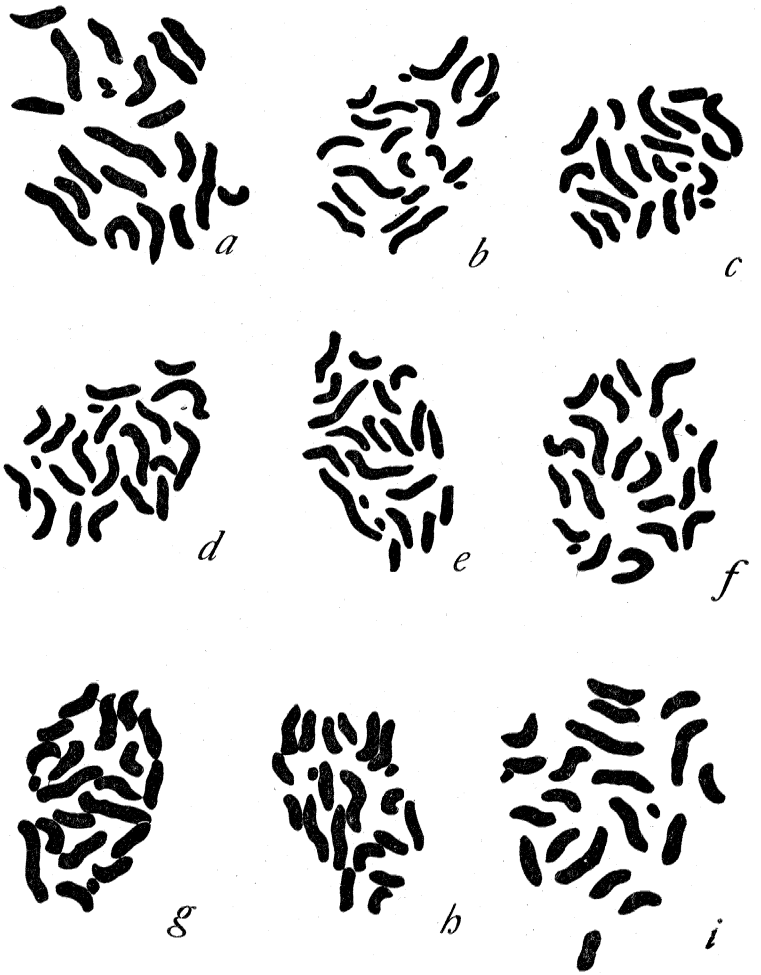

FIG. 10. *Chelinidea vittigera.* Chromosome-groups of embryonic cells. *a–h*, 21-chromosome type; *b*, *c*, *d*, *e* and *f*, from the same embryo; *a*, from an early cleavage. *i* exceptional group with twenty-two chromosomes.

In Fig. 10, *a–h*, are shown eight 21-chromosome groups. Fig. 10, *a*, is from an early cleavage stage corresponding approximately to the fourth cleavage of holoblastic eggs.[13] Fig. 10, *b*, *c*, *d*, *e*, and *f* are from an early blastoderm stage. Seven more perfectly clear counts were made in this embryo, all giving 21 chromosomes, making twelve in all. Fig. 10, *g* and *h*, are from another embryo in the same stage. Fig. 11 shows six of the 22-chromosome groups. Fig. 11, *a*, is taken from an early cleavage

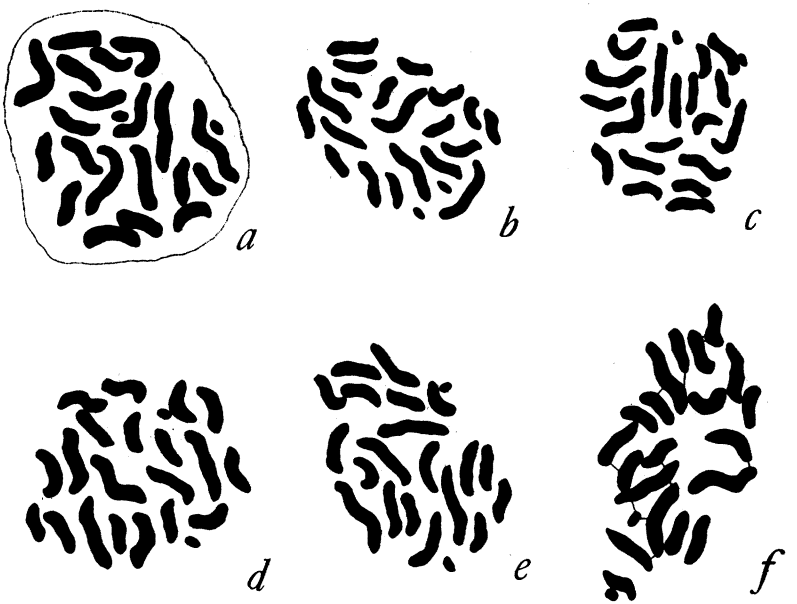

FIG. 11. *Chelinidea vittigera.* Chromosome-groups of embryonic cells, 22-chromosome type. *b*, *c*, *d* and *e*, from the same embryo; *f*, from another embryo; *a*, from an early cleavage.

stage, approximately the fourth. Fig. 11, *b*, *c*, *d* and *e*, are from an early blastoderm stage in which eleven perfectly clear groups were found all with 22 chromosomes. Fig. 11, *f*, is from another early blastoderm.

As in *Anasa*, it seems fair to conclude that the embryos with 21 chromosomes are males, those with 22 chromosomes, females,

[13]After the "second cleavage," the nuclei apparently do not divide quite synchronously so that one may find at times an odd number of nuclei, some resting and some in division.

for these groups correspond in number and size-relations with those of the spermatogonia and oögonia respectively. Like *Anasa* again, the male groups contain three chromosomes which are distinctly larger than the others (Fig. 10, *a–f* and *h*), one of them probably representing the unpaired idiochromosome. In the female groups four largest chromosomes can frequently be distinguished (Fig. 11, *b*, *c* and *f*), though they cannot be identified in all the figures, probably on account of fore-shortening. Two of these may be considered as the paired idiochromosomes, in place of the unpaired element of the male groups. The *m*-chromosomes are typically paired elements of both male and female groups. All the chromosomes as in *Anasa* are more elongated than in the gonads but still preserve the same size-relations. The groups shown in Figs. 10 and 11 were selected from a large number of clear preparations. Fig. 10, *i*, is a group of 22 chromosomes taken from an embryo in which all other counts gave 21. For this, the single exception of its kind observed, it is difficult to give a satisfactory explanation, though the same possibilities suggested in connection with the exception found in *Anasa* (vid. page 102) might also apply here. There were no neighboring groups from which the extra chromosome could have been derived.

4. *Protenor belfragei.*

The embryonic mitoses of this species are not quite so favorable for making chromosome counts as those of the two preceding forms, on account of the elongation of the chromosomes in the early and even late cleavage stages. In the blastoderm stage the chromosomes are more compact, but very few embryos were obtained at this time so that the results are very meagre. Fig. 12 shows two 13-chromosome groups (*a* and *b*) taken from an early blastoderm stage, and one 14-chromosome group (*c*) from another embryo in the same stage.

In the 13-chromosome groups (Fig. 12, *a* and *b*) a very large chromosome, unquestionably the unpaired idiochromosome, stands out clearly, being more than twice as large as any other. A second largest pair can also be readily identified and a smallest pair, the *m*-chromosomes.

In the 14-chromosome group (Fig. 12, *c*) there are two very large chromosomes equal in size, in place of the unpaired element of the first two groups. These are no doubt the paired idiochromosomes. There is also a next largest pair but the *m*-chromosomes cannot be identified with certainty.

A comparison of these embryonic mitoses (Fig. 12) with those of the gonads (Fig. 5, *a–d*) shows that the 13-chromosome groups are similar in the number and size-relations of their chromosomes

FIG. 12. *Protenor belfragei*. Chromosome-groups of embryonic cells. *a* and *b*, 13-chromosome type, from the same embryo. *c*, 14-chromosome type, from another embryo.

to those of the spermatogonia, the 14-chromosome group, to those of the oögonia. Though the results are too few to justify broad conclusions, it is most probable that the embryo with 13 chromosomes is a male, the one with 14 chromosomes a female, thus bringing *Protenor* in line with *Archimerus*, *Anasa* and *Chelinidea*.

IV. SUMMARY AND CONCLUSION.

Among the results described in this paper,[14] those of particular interest are as follows:

1. In *Archimerus*, *Anasa* and *Protenor* there is an odd or unpaired chromosome in the spermatogonia which in *Protenor* is distinguishable by its size. The oögonia contain in addition to this chromosome, a second chromosome of the same size. These observations are in agreement with those of Wilson, Montgomery and of Lefevre and McGill for the forms mentioned.

2. The chromosomes in the reduced female groups (polar or oöcyte divisions) show the same relative size differences as the corresponding pairs in the oögonia (particularly well shown in *Protenor*).

3. All the chromosomes divide in both polar divisions (proof

[14] A preliminary note giving the most important results was published in *Science* for December 31, 1909.

decisive in *Archimerus*, less complete in *Anasa* and *Protenor*). There are no peculiar or "lagging" chromosomes in either of these divisions.

4. The female pronucleus contains a group of chromosomes similar to that borne by a spermatozoön having the "accessory" or idiochromosome (directly proved in *Archimerus*).

5. At fertilization the reduced groups from each pronucleus are separately distinguishable and the chromosomes show the same size relations as those of the spermatocyte and oöcyte divisions. There are no nucleoli in either pronucleus.

6. In the cleavage and early blastoderm nuclei of *Archimerus*, *Anasa*, *Chelinidea* and *Protenor*, the chromosomes are perfectly distinct and can be counted as readily as those in the gonads. Two types of embryos are found, one having an odd and the other an even number of chromosomes, these numbers being respectively the same as occur in the spermatogonia and oögonia. Accordingly it seems fair to conclude that the former are males, the latter females, and it thus becomes possible to distinguish the sex of an embryo by counting its chromosomes.

7. The idiochromosomes behave exactly like the other chromosomes, in the oöcyte divisions, at fertilization and in the cleavage and early blastoderm stages. They never show any resemblance to nucleoli and in *Protenor* they can be identified in all stages with absolute certainty.

It will be seen that the results in general bear out the assumptions made by Stevens, Wilson and others regarding the number and behavior of the chromosomes in the maturation of the female and in the somatic cells. They give additional morphological support to theories of sex-production based upon the presence or absence of certain chromosomes and to the hypothesis of chromosome-individuality or "genetic continuity of chromosomes" as Wilson ('09*c*) more cautiously calls it.

V. Review and Discussion.

The literature on the maturation and early development of the eggs of insects and allied forms is very extensive, covering a period of over fifty years, but it is beyond the scope of the present paper to review it in detail except in so far as it concerns

the history of the chromatin in the early stages. Considered from this standpoint, the results briefly are as follows:

Diptera.—Apart from the earlier works of Weismann and Blochmann in which the chromosomes were not especially considered, there are no observations except those of Henking ('88 and '93) on *Musca vomitoria.* In the first paper Henking figured the cleavage spindles but did not determine the number of chromosomes. Moreover, the results are difficult to interpret because of the standpoint taken in regard to "free-nuclei-formation." In the later paper Henking summarizes his previous results but gives no new observations.

Lepidoptera.—Platner ('88) described briefly the maturation and early cleavage of the parthenogenetic and fertilized eggs of *Liparis dispar* but gave no figures and no account of the chromosomes. Henking ('90) described and figured the maturation, fertilization and early cleavage of *Pieris brassicæ.* He found the haploid number of chromosomes to be 14 in both polar spindles and in the female pronucleus, but did not accurately determine the diploid number, though in a later paper he gives the probable number as 28. The same author ('92) gave a brief account of the maturation and early cleavage of *Bombyx mori* and *Leucoma salicis* in which the haploid chromosome-group was stated to be "at least 12," in both species. The diploid number was not determined.

Neuroptera.—As far as I am aware, there are no observations on the maturation and cleavage of mitoses of the eggs of this group. Miss McGill's ('06) observations on *Anax junius* and *Plathemis lydia* were confined to the nuclear changes during the growth period of the oöcytes.

Coleoptera.—Wheeler ('89) observed the formation of the first polar spindle of *Leptinotarsa* (*Doryphora*) *decemlineata* but did not determine the number of chromosomes. Henking ('92) in *Agelastica alni* found the haploid number of chromosomes to be about 12 in both polar spindles and the diploid number, 24–30 in the cleavage spindles. He also observed the approximate number in *Lampyris splendidula*, *Adimonia tanaceti*, and *Donacia* (*sericea* L.?). In none of these, however, did he observe the diploid groups in the cleavage stages. The observations of

Giardina ('01) on *Dytiscus marginalis* and likewise those of Debaisieux ('09) on the same species were confined to the growth period of the oöcytes. Both authors describe a large chromatic mass in the nucleus of the oöcyte, distinct from the chromosomes, which appears to be eliminated just before the maturation divisions. The latter are not described.

Orthoptera.—The observations of Blochmann ('87) and Wheeler ('89) on *Blatta germanica* were not very extensive from our point of view. Neither author determined the number of chromosomes in the maturation spindles. Wheeler, however, gives a good figure of a cleavage spindle showing 10 chromosomes. Gutherz ('07) in a brief paper, described a chromosome-nucleolus in the oöcytes of *Pyrrhocoris* but found no such body in the somatic mitoses of *Gryllus domesticus*. He therefore questioned the occurrence of "heterochromosomes," maintaining that there were probably 20 chromosomes in the somatic cells of the last named species and no "heterochromosomes." However in his later papers ('08 and '09*a*) he abandoned this view, describing typical "heterochromosomes" in the spermatogonia and oögonia of *Gryllus* as in other Orthoptera, and stating further, that the somatic cells have the same number of chromosomes as the oögonia and spermatogonia respectively though he gave no observations in support of this last statement. The observations of von Baehr ('07) on the parthenogenetic egg of the phasmid, *Bacillus rossii*, though detailed in some respects, are not quite conclusive in regard to the number of chromosomes. The egg nucleus, just before the first polar division, contains 18–20 chromosomes many of which are tetrads. In anaphase the double nature of the daughter halves often becomes apparent. Moreover, there is one large tetrad in the first division which again appears in the second. The number of chromosomes in the latter division was not determined. In a recent paper Buchner ('09) has described in *Gryllus campestris* an irregular nucleolus-like structure, the "accessory body," which persists through the growth period of the oöcytes. It is derived from a similar body in the oögonia which he apparently considers identical with the "accessory" chromosome of other forms. The maturation mitoses were not observed. Gutherz ('09*b*) working on a nearly related species, *Gryllus domes-*

ticus, finds a similar body in the growth period of the oöcytes. After tracing its history, he concludes however that it is comparable to a nucleolus and is not to be confused with a "heterochromosome." Buchner's identification of the "accessory body" as a chromosome thus appears very doubtful.

Hymenoptera.—Blochmann's ('89) observations on the maturation of the fertilized and parthenogenetic egg of the bee were concerned chiefly with the number of polar bodies formed in the two sorts of eggs. Though the polar spindles were figured the exact number of chromosomes was not determined. Henking ('92) found the haploid number of chromosomes to be 10 in the polar spindles of *Lasius niger* and the diploid number in the cleavage spindles, 20. In the unfertilized egg of *Rhodites rosæ* he found 9 chromosomes in the polar spindles. In the cleavage nuclei the number was 18–20, *i. e.*, the number of chromosomes in the female pronucleus had been doubled. Petrunkewitsch ('01) working on the fertilized and parthenogenetic eggs of the bee, found that the first polar division was equational, the number of chromosomes being 16. In the second division there occurred in both sorts of eggs, a reduction of the chromosome-number to about half, *i. e.*, from 16 to 8. In the parthenogenetic (drone-) egg, the female pronucleus contained at first 8, but later 16 chromosomes, the latter being produced by a doubling of the haploid group, so that in the equatorial plate of the first cleavage spindle the diploid number, 16, again appeared. In later cleavages there was a progressive doubling of the chromosome-number, producing multiple groups of 32 and 64. Silvestri ('06 and '08) has described in detail the maturation, fertilization and cleavage of several species of parasitic hymenoptera (*Litomastix*, *Encyrtus*, *Oöphthora*, *Ageniaspis*). However, since his results do not include the determination of the exact number of chromosomes in the early stages, it will be unnecessary to review them here. Doncaster's ('07) results on *Nematus ribesii* (Tenthredinidæ) are quite anomalous and difficult to interpret. He finds that there are two types of maturation in the female. In some eggs there is no reduction of chromosomes, the female pronucleus receiving the diploid number, 8. In others typical reduction occurs, the egg nucleus receiving in all probability the haploid number, 4.

The former type of egg develops parthenogenetically, the latter only being capable of fertilization, it is supposed. In some somatic tissues, such as the ovary sheath, there are more than the diploid number of chromosomes, as in the bee and in *Ascaris*. In a later brief communication, however, Doncaster ('10*a*) states that his observations on the polar mitoses may require revision and that the behavior of the chromosomes in *Nematus ribesii* is so difficult to follow that it is doubtful if a satisfactory interpretation can be obtained in this species. In a very recent paper, the same author ('10*b*) describes in detail the maturation, fertilization and early cleavage of *Neuroterus lenticularis* (Cynipidæ). Here again some of the results are quite novel. The mitoses of the primitive ova found in young female larvæ of the summer generation contain about 20 chromosomes, like those of the somatic cells. In the maturation of the summer eggs apparently two divisions occur, the female pronucleus probably containing 10 chromosomes. The eggs are fertilized and in the cleavage spindles about 20 chromosomes appear. The results on the maturation of the spring (parthenogenetic) egg are so anomalous that it seems best to quote from Doncaster's own summary (*loc. cit.*, p. 102): "The maturation of the spring egg has not yet been sufficiently studied, but it appears that some eggs undergo at least one maturation division, others probably none. In eggs in which maturation has occurred segmentation mitoses show 10 chromosomes; all the eggs laid by one individual female in which the chromosomes could be counted were of this type, and it is suggested that these develop into males. In the eggs laid by other females, however, 20 chromosomes appear in the segmentation divisions; in these, polar chromosomes appear to be absent, and it is probable that there has been no maturation division, and that these eggs would develop into females." It will be seen that no definite conclusions can be drawn without further confirmatory observations. Schleip's ('08) observations on the polar body formation in *Formica sanguinea* were confined chiefly to the parthenogenetic egg. He found in the latter the haploid number of chromosomes to be about 24 in the maturation spindles and female pronucleus. This number also appears in the first cleavage nucleus. In the fertilized egg, the number of chromosomes

in the male and female pronuclei was not determined with certainty but is probably 24 in each. On the whole, the chromosomes were small and the size-differences not well marked.

Hemiptera-homoptera.—The older papers of Weismann, Witlaczil, Blochmann and Will on the early embryology of aphids contain no detailed account of the chromosomes. Stschelkanovzew ('04) in a brief paper on the maturation and early cleavage of the summer (parthenogenetic) egg of *Aphis rosæ*, gave 14 as the number of chromosomes in the maturation spindle (only one polar body is formed). In one first cleavage spindle there were only 11 chromosomes but he considered that three of these might be double elements, thus giving the diploid number, 14, in both maturation and cleavage. Miss Stevens ('05*a*) and Hewitt ('06),[15] however, found the diploid number in the parthenogenetic egg of *Aphis rosæ* to be 10 and this count has been confirmed by von Baehr ('09) in the same species. Miss Stevens also found that the winter (fertilized) egg gave off two polar bodies in which the haploid number, 5, was present. Three of these authors observed marked size differences in the chromosomes of the maturation and early cleavage stages, both Miss Stevens and von Baehr finding four smallest chromosomes constantly. Miss Stevens ('06*b*) in a very extensive paper described the maturation and cleavage in a large number of aphids, with especial reference to the number and behavior of the chromosomes. Without giving a detailed review of her results it may be said in general that the number and size differences of the chromosomes was found to be constant for the species and that this constancy applies to the diploid groups whether in the maturation spindle of a parthenogenetic egg or in its cleavage spindles. In many cases also the haploid group was found to exhibit the same relative size differences as the diploid group of the same species. In the fertilized egg of the "Goumi aphid," the number of chromosomes in the male and female pronuclei just before copulation was shown to be 5 in each, *i. e.*, the haploid number. Miss Stevens concluded from her observations on aphids up to this time that there were no "heterochromosomes" in this group, but

[15]Hewitt's results are known to me only through the brief mention made by von Baehr ('09, p. 285).

more recently ('09) she has abandoned this view and has reached conclusions in agreement with those of Morgan and von Baehr, mentioned below. The results of Tannreuther ('07) on the maturation and cleavage of several species of aphids differ in many important respects from those of other workers on the same group. They have been questioned by Morgan ('09) and von Baehr ('09) and will not be considered here. Morgan ('08 and '09) has traced the full history of the chromosomes through several generations of phylloxerans. He observed that the parthenogenetic eggs of the second generation, *i. e.*, those which produce the sexual individuals, are of two sorts both as to their size and the number of chromosomes in the embryos which they produce. The male embryos have actually two less chromosomes than the female though this difference is not always apparent owing to fusions occurring between certain chromosomes (the "accessories.") The embryonic chromosome-groups of female individuals contain four "accessory" chromosomes. Those of the males, however, have but two "accessories" since although males are produced parthenogenetically from females, two of these chromosomes are given off to the polar body in the maturation of the male-producing egg. The most important conclusions to be drawn from these results for our purposes are, that idiochromosomes are present throughout the entire life-cycle and that it is possible to diagnose the sex of an embryo by counting its chromosomes, though here it is true, sex is also associated with the size of the egg. Von Baehr ('09) described the maturation and cleavage of the parthenogenetic eggs of several species of aphids. His results were in general similar to those of Miss Stevens, the maturation and cleavage mitoses being similar in the number and size relations of the chromosomes. He did not observe any elimination of "accessory" chromosomes in the polar division of male eggs as in the phylloxerans. However, in one maturation spindle of *Aphis saliceti* (*loc. cit.*, Pl. XIV., Fig. 42) he figures 5 chromosomes, the remaining figures showing 6, and in a male somatic cell (Pl. XV., Fig. 94), as well as in the spermatogonia, 5 chromosomes again appear. Moreover his results on the spermatogenesis of *Aphis saliceti* as well as those of Miss Stevens ('09) on the spermatogenesis of other aphids would seem

to indicate that the behavior of the chromosomes in the female line is probably similar to that in phylloxerans.

Hemiptera-heteroptera.—The only observations on the maturation and cleavage of the egg in this group are those of Henking ('92) on *Pyrrhocoris*. He has given a very extensive and detailed account of the chromosome history in this form and in a previous paper ('91) described the spermatogenesis. He found that in the diploid groups of the oögonia, there were 24 chromosomes. The follicle and connective tissue cells, both larval and adult also showed this number. In the haploid group of the first polar spindle 12 dumbbell-shaped chromosomes appeared. In one such group ('92, Pl. III., Fig. 83) one chromosome is much larger than the rest and is probably the idiochromosome pair (cf. Wilson's ('09*d*) figures of the oögonial groups). The second polar spindle showed again 12 dumbbell-shaped chromosomes. The number of chromosomes in the male and female pronuclei was not accurately determined but in Henking's Fig. 90 (Pl. III.) one such nucleus shows 12 chromosomes. The early cleavage spindles were figured but of them the author says (*loc. cit.*, pp. 29–30): "The number (of chromosomes) cannot be accurately determined on account of the smallness of the spindle and the close grouping of the chromosomes . . . it should be 24." He thus did not distinguish two classes of embryos with reference to the chromosome number. This, no doubt, was partly due to the fact that he had not observed any difference in the number of chromosomes in the spermatogonia and oögonia and did not appreciate the significance of the idiochromosome ("accessory" chromosome) which he himself was the first to describe. Foot and Strobell ('09) have described the growth period of the oöcytes of *Euschistus variolarius*. In accordance with the earlier account of Wilson ('06), they find no chromatin nucleolus in the young oöcytes or germinal vesicles of this species but in the older oöcytes and in the germinal vesicles there is a relatively large achromatic nucleolus. The maturation divisions were not described.

Arachnida.—Montgomery's ('07) results on *Theridium* are not very extensive from the chromosome-standpoint. He found in the second polar spindle 12 chromosomes and in a fourth cleavage spindle 24 chromosomes. No idiochromosomes were observed.

Excluding the early cleavage groups mentioned above, a number of authors have described the chromosomes of older somatic cells. Henking ('92) found the number of chromosomes in the egg-follicle and connective tissue cells, of *Pyrrhocoris* to be 24. Petrunkewitsch ('01) observed that young blastoderm-cells of the bee contain multiple groups. Miss Stevens ('05*b* and '06*b*) described the somatic groups of several species of Coleoptera and found that the small idiochromosome which occurs only in the male of these forms could be readily identified. Von Baehr ('09) observed that the male somatic groups of *Aphis saliceti* contain 5 chromosomes, one less than the female. Gutherz ('09*a*) concluded that in *Gryllus domesticus* the somatic cells have the same number of chromosomes as the oögonia and spermatogonia respectively, though his observations on this point were not very extensive and no figures of somatic mitoses were given. Doncaster ('10) observed the somatic groups in the male and female pupæ of the gall-fly. He found that in the male, some somatic mitoses show the diploid number of chromosomes while others may show the haploid number. In the female, all somatic mitoses have the diploid number. The very anomalous conditions described for the male do not at present rest upon demonstrative evidence as the chromosomes were found to be small and difficult to count. In addition to the above-mentioned observations, most recent papers on the spermatogenesis of insects contain accounts of the oögonial groups in which idiochromosomes can often be identified.

From the foregoing brief view of the literature on the chromosomes in oögenesis and cleavage, it is evident that with the exception of Miss Stevens and Morgan none of the authors have traced the idiochromosomes into the cleavage and later somatic mitoses, and none but Miss Stevens, Morgan and von Baehr have shown that the embryonic or larval somatic cells of male individuals differ from those of females in the number or size of their chromosomes. Morgan has also shown that idiochromosomes are present in the polar spindle where, in his material, they behave in a characteristic manner. The results on the whole show, I think, that idiochromosomes ("heterochromosomes") are constant chromosome-elements and not merely temporary structures (nucleoli) present during maturation.

Outside of the air-breathing arthropods, there are, as mentioned before, two other groups in which idiochromosomes or similar structures have been found in maturation and cleavage. Baltzer ('08) has found that in two species of sea-urchins there is a particular hook-shaped chromosome which occurs in only a part of the mature eggs. The eggs are thus of two types with respect to this element. (It is replaced by a chromosome of the ordinary sort in the eggs which lack it.) The sperm nuclei on the contrary are all alike. It is not improbable, Baltzer concludes, that the determination of sex depends upon this dissimilarity of egg nuclei, and therefore *lies with the female* (*i. e.*, with the egg), as in the male and female (parthenogenetic) eggs of aphids and phylloxerans. The peculiar hook-shaped elements might thus be called "idiochromosomes." Eggs which contain this element would develop into females, those without, into males. In a very recent paper Boveri and Gulick ('09) have described briefly the chromosome-cycle in *Heterakis*, a nematode. Its cycle corresponds exactly with that of *Protenor* as given by Wilson ('06). The diploid number in the male (spermatogonia) is 9. During spermatogenesis the odd chromosome goes undivided to one pole of the spindle in the first spermatocyte division but divides in the second. The spermatozoa are thus of two classes, with 5 and 4 chromosomes respectively. The diploid number in the female was not determined with certainty but the haploid number in the germinal vesicles and polar spindles was found to be 5. The eggs are thus all alike and, it is assumed, will develop into males or females according as they are fertilized by 4-chromosome or 5-chromosome spermatozoa. The chromosomes of the cleavage nuclei were not described.

Since the results here described for coreid Hemiptera do not give any further insight into the fundamental question of sex-determination but only render the data more complete, it seems needless to add a lengthy discussion on this point. In the recent papers of Wilson, Bateson Castle, Boveri and Morgan, the cytological evidence relating to sex-determination has been thoroughly analyzed. It may be pointed out, however, that apart from theoretical considerations this evidence has been questioned from the standpoint of fact by several workers who have supported their

contentions by direct observations on insect spermatogenesis. Arnold ('08) from his observations on the spermatogenesis of *Hydrophilus piceus* concluded that there were no idiochromosomes in that form, although Miss Stevens has found these elements in all the Coleoptera which she has examined (42 species). The objection offered by Foot and Strobell ('07) to the presence of an odd number of chromosomes in the spermatogonia of *Anasa tristis*, hence of an "accessory" chromosome and the replies to this objection have already been considered. The present work, particularly the section dealing with the cleavage and early blastoderm nuclei, gives further proof that in this species, as well as in the other three examined, the number of somatic chromosomes in the male is one less than in the female. Gross, from his studies on *Syromastes marginatus* ('04) and *Pyrrhocoris apterus* ('06), concluded that the "accessory" chromosome could have no effect on sex-production in these two forms, for he believed that the number of chromosomes is the same in both sexes—22 in *Syromastes* and 24 in *Pyrrhocoris*. In the last named form his counts agree with the earlier ones of Henking ('91), though the latter was uncertain of the spermatogonial number. Recently however Wilson ('09*b*, '09*d*) has reëxamined both these forms and finds that in *Pyrrhocoris*, the male has one less chromosome than the female, *i. e.*, 23 instead of 24, while in *Sryomastes*, the male has 22 as described by Gross but the female has 24 instead of 22. *Pyrrhocoris* may thus be plaecd in the same class with such forms as *Archimerus*, *Anasa* and *Protenor*. *Syromastes*, however, is unique among the Hemiptera heteroptera in having a bivalent "accessory," though a similar condition has been described by Morgan ('09) in the homopteran, *Phylloxera caryæcaulis*, while Payne ('09) describes several cases among the Reduviidæ (*Fitchia*, *Rocconota*, *Conorhinus*) in which the large idiochromosome (which represents the "accessory") is double.

In addition to the objections cited above, a number of authors have either expressed their doubts of the presence of two sorts of spermatozoa, or, while admitting the existence of such a dimorphism, have questioned its sexual significance. In recent years, however, evidence has been steadily accumulating in support of the conclusion that a nuclear dimorphism of the sperm—

or of the eggs—does occur, not only in insects but in at least two other groups of animals and that it bears a definite relation to sex-production. Whether the sexual tendencies are carried by specific chromosomes or whether certain combinations of chromosomes cause one sex or the other to arise, is still an open question, but that it is possible to demonstrate a nuclear difference in the gametes, and frequently indeed, in the somatic tissues of the two sexes, is now, I think, placed beyond doubt by many decisive observations.

April 27, 1910.

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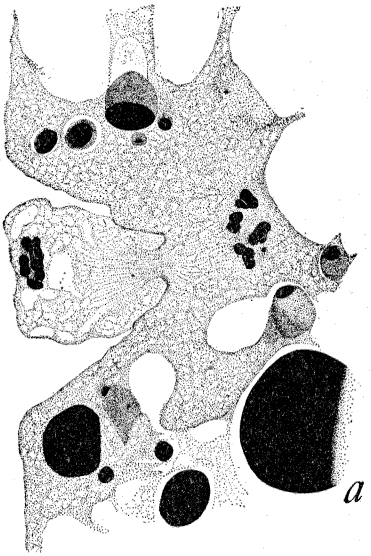
Explanation of Plate I.

Archimerus alternatus. *a*, formation of the first polar body—the mitotic figure is in final anaphase, the inner daughter group showing eight chromosomes; the cytoplasm contains a number of yolk spheres of different sizes and staining capacity. *b*, formation of the second polar body—the inner group of chromosomes have been transformed into the female pronucleus; those of the outer group are still separate. *c*, the female pronucleus advancing into the egg in its cytoplasmic "island"; the latter also contains two yolk spheres. *d*, the sperm-head advancing into the egg, in its cytoplasmic "island"; it is preceded by an aster and surrounded by a clear area. The magnification is 1,375 diameters.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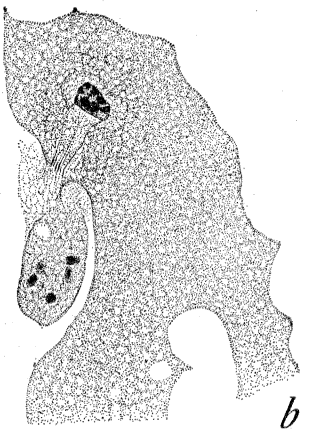
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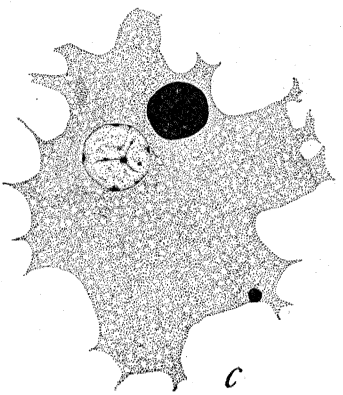
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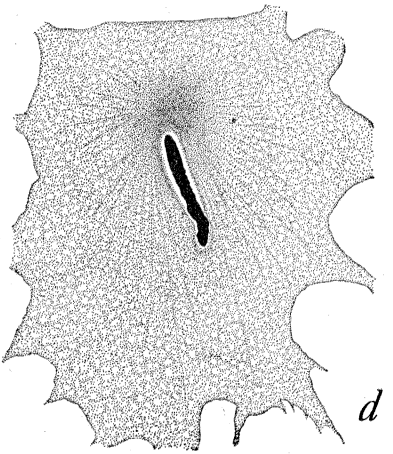
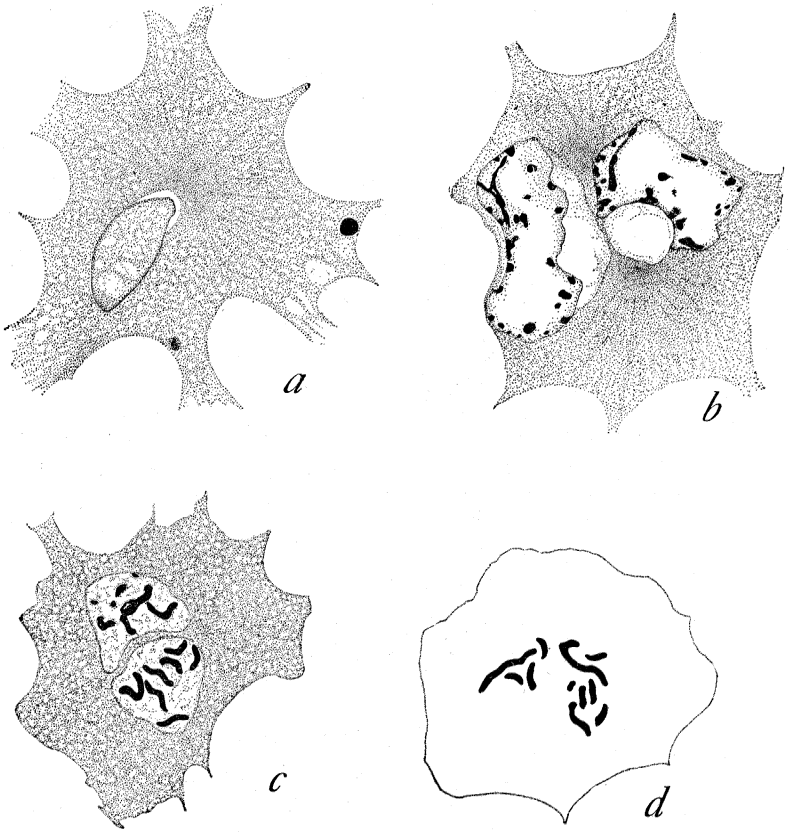

d

Explanation of Plate II.

a–c, Archimerus alternatus. a, a stage letter than Pl. I., *d*—the sperm head has been transformed into the male pronucleus and is still advancing into the egg preceded by its aster and a small clear area. *b*, an early stage in the copulation of the male and female pronuclei—each is in contact with a clear area and an amphiaster lies between them. *c*, a later stage in the copulation of the pronuclei—the nuclear membranes are still intact; seven chromosomes appear in the lower nucleus, the *m*-chromosome being absent; a faint aster appears on the right. *d, Protenor belfragei:* First cleavage prophase, polar view—the male and female groups are still separate, one of them being incomplete; an idiochromosome appears in each group. The magnification is 1,375 diameters.


a
b
c
d